

# Positive Relationship of the Avian Leukosis-J Strain Virus to the Detection of *Campylobacter* in the Digestive Tract and Semen of Broiler Breeder Roosters

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**Primary Audience:** Production Managers, Hatchery Managers, Field Service Personnel,  
Flock Supervisors, Veterinarians

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## SUMMARY

At 45 wk of age, 99 roosters housed in individual cages were tested for avian leukosis-J strain virus (ALV-J); 32 tested positive and 67 tested negative. These roosters were fed a restricted diet to maintain weight to primary breeder specifications. Individual semen and ceca samples were analyzed for presence and level of *Campylobacter*. Population levels of *Campylobacter* in semen and ceca of ALV-J individuals were 3.5 times higher than negative birds. Controlling viruses such as ALV-J may be an important part of limiting colonization of broiler breeders by organisms such as *Campylobacter*.

**Key words:** campylobacter, semen, digestive tract, avian leukosis-J strain virus

2004 J. Appl. Poult. Res. 13:44–47

## DESCRIPTION OF PROBLEM

The avian leukosis-J strain (ALV-J) virus is a vertically transmitted virus that emerged among the world's primary broiler breeder lines of chickens during the early 1990s. The ALV-J has received much attention in recent years because its presence in parent breeding flocks can result in increased mortality (as high as 40% of male and female breeders), reduced fertility, and decreased egg weight and shell quality of fertile hatching eggs.

Also receiving much attention in recent years are foodborne human pathogens associated with poultry. One of the most important bacteria causing human foodborne illness is *Campylobacter*. Campylobacteriosis has been closely associated with the consumption of undercooked commercial poultry, and at present the sources of introduction of this bacteria into broiler flocks are unknown. In the past few years, it has been shown that *Campylobacter* can pass from the breeder (parent) to the broiler

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TABLE 1. Rate and level of naturally occurring *Campylobacter jejuni* from the reproductive (semen) and digestive tracts (ceca) of healthy and infected broiler breeder roosters

Type of samples	Condition of roosters <sup>A</sup>			
	Healthy		Infected with ALV-J <sup>B</sup>	
		<i>Campylobacter</i> (cells/mL or /g)	n of <i>Campylobacter</i> positive/n sampled	<i>Campylobacter</i> (cells/mL or /g)
Semen	7/67 (10.6%) <sup>a</sup>	25	5/20 (25%) <sup>b</sup>	79
Ceca	20/67 (30%) <sup>a</sup>	3,981	15/28 (53.6%) <sup>b</sup>	19,953

<sup>a,b</sup>Values within the same row are significantly different as determined by chi-square test for independence.

<sup>A</sup>Values calculated using values from *Campylobacter* (+) samples.

<sup>B</sup>Avian leukosis-J strain virus.

(offspring) through the fertile egg [1, 2]. *Campylobacter* is found occurring naturally in all segments of the reproductive tract of actively laying breeder hens [3, 4, 5] and in the semen of commercial broiler breeder roosters [6, 7, 8]. The presence of naturally occurring *Campylobacter* in the reproductive and digestive tracts of male and female breeders and transmission of this bacterium to the fertile egg must be considered as potential sources for *Campylobacter* contamination of market poultry. The objective of this study was to determine if the prevalence of ALV-J in mature roosters affected the incidence and level of *Campylobacter* shed from the reproductive tract (semen) and the digestive tract (ceca) of broiler breeder roosters.

## MATERIALS AND METHODS

At 45 wk of age, 99 broiler breeder males were individually caged in an environmentally controlled research facility 77 m (length) × 12 m (width) at the University of Georgia's Poultry Research Center. The 32 roosters that tested positive [9] for ALV-J were housed closest to the exhaust fans. The remaining roosters, which tested negative for the ALV-J, were on the opposite side of the facility to reduce the likelihood of viral spread. ALV-J-negative roosters were also fed and handled first to decrease the chances of disease spread. Prior to the start of this study, the cockerels were maintained to primary breeder weight specifications. Adult roosters were abdominally massaged, and individual semen samples were aspirated into clean disposable test tubes 2 d prior to being killed at 59 wk of age. Semen samples

were collected before feeding to reduce the presence of fecal contamination. Semen sample tubes were placed on ice during collection and transportation to the laboratory. Approximately 3 mL of avian semen extender [10] were added for every ≤1 mL of semen prior to bacterial culturing. An aliquot (0.1 mL) of this mixture was plated directly onto Campy-cefex agar plates in duplicate and incubated at 42°C for 48 h in a microaerobic atmosphere (5% O<sub>2</sub>, 10% CO<sub>2</sub>, 85% N<sub>2</sub>). Characteristic colonies were confirmed as *Campylobacter* by oil immersion microscopic observation of spiral morphology and darting motility from prepared wet mounts. Selected isolates were also confirmed using a commercial latex agglutination kit specific for *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter lari* [11]. To enhance the detection of low numbers of bacteria, semen samples were also enriched by inoculating 0.1 mL of undiluted semen into a reduced volume (5 mL) of Bolton broth and incubated for 48 h in a microaerobic environment. Enriched samples were plated onto Campy-cefex agar as previously described.

For collection of ceca, all roosters were humanely killed by cervical dislocation. The ceca were then aseptically removed and mixed with phosphate-buffered saline (pH 7.2 at 1:3 wt/vol) by using a stomacher. Serial dilutions of cecal suspensions were surface plated onto Campy-cefex agar and incubated microaerobically at 42°C for 24 to 48 h [12]. Suspect colonies were selected and confirmed as previously described. Samples were recorded as positive or negative for *Campylobacter*. The analysis of data was calculated with a statistical pro-

gram [13] by using the chi-squared test for independence.

## RESULTS AND DISCUSSIONS

Two separate groups of individually caged commercial broiler breeder roosters (one group was healthy roosters, and the other group was roosters that tested positive for ALV-J) were tested for the presence of *Campylobacter* at 59 wk of age. Individual semen samples were analyzed from each rooster when possible, and later ceca were analyzed from each rooster. The semen samples (10.6%) taken from healthy roosters a few days before euthinazation were found to have an average of 25 *Campylobacter* organisms per milliliter of semen (Table 1). In the group infected with ALV-J, 5 of the 20 (25%) infected roosters were shedding an average of 79 *Campylobacter* cells per milliliter of semen. Only 20 of the birds infected with ALV-J yielded a semen sample when abdominally massaged. For the healthy chickens, 30% (20/67) were found to have *Campylobacter* in their ceca, with an average of approximately 4,000 *Campylobacter* per gram of ceca. In the group infected with ALV-J over 50% (15/28) of the

roosters had *Campylobacter* in their ceca with an average level of about 20,000 *Campylobacter* per gram of ceca. Four of the 32 infected birds died prior to sampling of the ceca. Our findings indicate that ALV-J infection in late-life broiler breeder roosters is associated with increased incidence of *Campylobacter* in the digestive and reproductive systems and as a result may increase transmission of *Campylobacter* to a breeder hen's reproductive tract and subsequently to fertile eggs and newly hatched chicks [6, 7, 8].

Prevention and control strategies of important foodborne pathogens such as *Campylobacter* must be properly designed and implemented at all levels of the production cycle. The presence of *Campylobacter* in chicken semen and throughout the reproductive systems of male and female breeders suggests that reproductive activities and the resultant fertile eggs can impact the safety of market poultry. The findings of this study show that the presence of a ALV-J infection increases *Campylobacter* colonization of the intestinal tract and the level of contamination of the semen in breeder roosters.

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## CONCLUSIONS AND APPLICATIONS

1. *Campylobacter* was readily isolated from the ceca (30% of birds negative for ALV-J and 54% of birds positive for ALV-J) and semen (10.6% of birds negative for ALV-J and 25% of birds positive for ALV-J birds) of commercial broiler breeder roosters.
  2. An infection with ALV-J in late-life broiler breeder roosters resulted in prevalence of *Campylobacter* in ceca and semen of these roosters.
  3. If prevention and control strategies for bacteria such as *Campylobacter* are to be properly designed and effectively implemented, then the effect of viral infection on its prevalence and numbers in the reproductive and intestinal tracts must be considered.
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### ***Acknowledgments***

Outstanding technical assistance was received from D. P. Posey and S. C. Mize.